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(19)



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European Patent Office

Office européen des brevets



(11)

EP 0 870 827 A2

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
14.10.1998 Bulletin 1998/42

(51) Int. Cl.⁶: **C12N 15/12, C07K 14/705,
C07K 16/28, A61K 38/17,
C12Q 1/68, G01N 33/68**

(21) Application number: **97310562.0**

(22) Date of filing: **23.12.1997**

(84) Designated Contracting States:
**AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE**

(30) Priority: **14.03.1997 US 41230 P
09.05.1997 US 853684
22.08.1997 US 916625**

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(54) **Tumor necrosis factor related receptor, TR6**

(57) TR6 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR6 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease., among others and diagnostic assays for such conditions.

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Description

This application is a continuation-in-part application of U.S. Serial No: 08/853,684, filed May 9, 1997, which claims the benefit of U.S. Provisional Application No: 60/041,230, filed March 14, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Tumor Necrosis Factor Related family, hereinafter referred to as TR6. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counterligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF- α , lymphotoxin- α (LT- α , also known as TNF- β), LT- β (found in complex heterotrimer LT- α 2- β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF)). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., *Biologicals*, 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., *supra*).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., *Nature* 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., *Science* 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innervation of peripheral structures (Lee, K.F. et al, *Cell* 69:737 (1992)).

TNF and LT- α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT- α , acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT- α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., *Science* 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., *Cell* 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TR6 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TR6 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TR6 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TR6 activity or levels.

DESCRIPTION OF THE INVENTION

15 Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TR6" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

20 "Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said TR6 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TR6.

"TR6 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

25 "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

30 "Polynucleotide" generally refers to any polyribonucleotide or polydeoxiribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

35 "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation,

iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to TR6 polypeptides. The TR6 polypeptides include the polypeptides of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within TR6 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably TR6 polypeptides exhibit at least one biological activity of the receptor.

The TR6 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the TR6 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TR6 polypeptides. As with TR6 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TR6 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TR6 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TR6 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to TR6 polynucleotides. TR6 polynucleotides include isolated polynucleotides which encode the TR6 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, TR6 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 1 encoding a TR6 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS: 1 and 3. TR6 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length, and a polynucleotide that is at least 80% identical to that having SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under TR6 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplifica-

tion or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such TR6 polynucleotides.

TR6 of the invention is structurally related to other proteins of the Tumor Necrosis Factor Related family, as shown by the results of sequencing the cDNA encoding human TR6. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide numbers 94 to 1329) encoding a polypeptide of 411 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 58% identity (using GAP (From GCG)) in 411 amino acid residues with DR4, the receptor for the ligand TRAIL (Pan,G., O'Rourke,K., Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 70% identity (using GAP (from GCG)) in 1335 nucleotide residues with DR4, the receptor for the ligand TRAIL. TR6 contains a death domain (amino acids 290 to 324 in SEQ ID NO:2) which is 64% identical to the death domain of the human Death receptor 4 (DR4) (Pan,G., O'Rourke,K., Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)), 35.7% identical to the death domain of the human Death receptor 3 (DR3) (A.M. Chinnaiyan, et al, Science 274 (5289), 990-992 (1996)), 32.7% identical to the death domain of human TNFR-1, and 19.6% identical to the death domain of CD95 (Fas) (I. Cascino, J. Immunol. 154 (6), 2706-2713 (1995)).

Table 1^a

1	CTTTGCGCCC ACAAATACA CGACGATGC CGATCTACT TTAAGGGCTG
51	AAACCCAOGG GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC
101	AACGGGGACA GAAOGCCCOG GCOGCTTCGG GGGCCCGGAA AAGGCAOGGC
151	CCAGGACCCA GGGAGGOGOG GGGAGCCAGG CCTGGGCCCC GGGTCCCCAA
201	GACCCTTG TG CTGGTTGT OG COGOGTCTT GCTGTTGGTC TCAGCTGAGT
251	CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCA
301	CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAC CTGGACACCA
351	TATCTCAGAA GACGGTAGAG ATTGCATCTC CTGCAAATAT gGACAGGACT
401	ATAGCACTCA aTGAATGAC CTCCTTTTCT GCTTGCGCTG CACCAGGTGT
451	GATT CAGGTG AAGTGGAGCT AAGTCCCTGC ACCACGACCA GAAACACAGT
501	GTGT CAGTGC GAAGAAGCA CCTTCOGGA AGAAGATTCT CCTGAGATGT

5

551 GCOGGAAGTG COGCACAGGG TGTCCCagAG GGATGGTCAA GGTGGTGAT

10

601 TGTACACCCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT

15

651 CATAgGAGTC ACAGTTGCAG CGTAGTCTT GATTGTGGCT GTGTTTGT

20

701 GCaAgTCTTT ACTGTGGAag AAAGTCCTTC CTTACCTGAA AGGCATCTGC

25

751 TCAGTGGTG GTGGGGACCC TGAGGTGTG GACAGAAGcT CACAACGACc

30

801 TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC TTGCAGCCCA

35

851 CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA

40

901 GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAAACGGC

45

951 AGAAGCTGAA AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG

50

1001 GTGATCCCAC TGAGACTCTG AGACAGTGCT TCGATGACTT TGCAGACTTG

1051 GTGCCCTTTG ACTCCTGGGA gCgCTCATG AGGAAGTTGG GCCTCATGGA

1101 CAATgAGATa aaGGTGGCTA AAGCTGAGGC AGGGGCCAC AGGGACACCT

1151 TGTACAGAT GCTGATAAAG TGGGTCAACA AAACGGGGCG AGATGCCTCT

1201 GTCCACACCC TGCTGGATGC CTTGGAGAGC CTGGGAGAGA GACTTGCCAA

1251 GCAGAAGATT GAGGACCACT TGTGAGCTC TGGAAAGTTC ATGTATCTAG

1301 AAGGTAAATGC AGACTCTGCC ATGTCCTAAG TGTGATTCTC TTCAGGAAGT

1351 CAGACCTTCC CTGGTTTACC TTTTTTCTGG AAAAAGCCCA ACTGGACTCC

1401 AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACGGTAC TGGAAGAAAC

1451 TCTCCCATCC AACATACCCC AGTGGATGGA ACATCCTGTA ACTTTTCACT

1501 GCACTTGGCA TTATTTTAT AAGCTGAATG TGATAATAAG GACACTATGG

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1551 AAATGTCTGG ATCATTCCGT TTGTGCGTAC TTTGAgATTT GGT TTGGGAT
 1601 GTCATTGTTT TCACAGCACT TTTTATCCT AATGTAAATG CTTTATTAT
 1651 TTATTTGGGC TACATTGTAA gATCCATCTA CACAGTGGT GTCCGACTTC
 1701 ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGG TGCGGGGCAg
 1751 TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA
 1801 TAGCCTTGAC CTCTCAGGCT CAAGCGATTCT CCCACCTCA GCCATCCAA
 1851 TAGCTGGGAC CACAGGTGTG CACCACCAG CCGGCTAAT TTTTGTATT
 1901 TTGTCTAgAT ATAGGGGCTC TCTATGTTGC TCAGGTGGT CTGgAATTCC
 1951 TGGActCAAG CAGTCTGCCC AOctCAGAct CCCAAAGGG TGGAAATTAGA
 2001 GGCGTGAGCC CCCATGcTTG gCCTTAOctT TcTACTTTTA TAATTCTGTA
 2051 TGTATTATT TTATGAACAT GAAGAACTT TAGTAAATGT ACTTGTTTAC
 2101 ATAGTTATGT GAATAGATTA GATAACATA AAAGGAGGAG ACATACAATG
 2151 GGGGAAGAAG AAGAAGTCCC CTGTAAGATG TCACTGTcTG GGTCCAGCC
 2201 CTCCCTCAGA TGTACTTTGG CTTCAATGAT TGGCAACTTC TACAGGGGCC
 2251 AGTCTTTTGA ACTGGACAAC CTTACAAGTA TATGAGTATT ATTTATAGGT
 2301 AGTTGTTTAC ATATGAGTcG GGACCAAAGA GAACTGGATC CAOGTGAAGT
 2351 CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TcTCATTTGC ACCCATAGCC
 2401 CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA
 2451 ACAATAGGGT CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTGGGCT
 2501 CAGGAGATAG AAGACAAAT CTGTCTCCCC ACGTCTGCCA TGGCATCAAG
 2551 GGGGAAGAGT AGATGGTGCT tGAGAATGGT GTGAAATGGT TGCCATCTCA

5

2601 GGAGTAGATG GCCGGCTCA CTTCTGGTTA TctGT CACCC TGAGCCCAtG

10

2651 AGCTGCCTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCGCTCTG

15

2701 TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC

2751 AGGACCTAGA ATGGCTGACG CATTAAAGTT TTCTTcTTGT GTCTGTCT

20

2801 ATTATGTTT TAAGACCTCA GTAACCATT CAGCCTCTT CCAGCAAACC

2851 CTTCTCCATA GTATTT CAGT CATGGAAGGA TCATTTATGC AGGTAGTCAT

25

2901 TCCAGGAGTT TTTGGTCTT TCTGTCTCAA GGCATTGTGT GTTTTGTCC

2951 GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATcAcACATT

30

3001 CAGACTGTtG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTtCTGGT

3051 CTTtGcAcTT CCATCcttC CCAcTTCCAT cTGGCATCCC CAOGcGTTGT

35

3101 CCCcTGCAcT TcTGGAAGGC ACAGGGTGCT GCTGCTT CCT GGTCTTGCC

3151 TTTGCTGGGC cTTCTGTGCA GGAOGCTCAG CCTCAGGGCT CAGAAGGTGC

40

3201 CAGTCGGTC CCAGTCCCT TGTCCCTCC ACAGAGGCCT TcTAGAAGA

3251 TGCATCTAGA GTGT CAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT

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3301 TAATTTTTTT GAGACAGAAT CTCACTCTCT GCGCCAGGCT GGAGTGCAAC

3351 GGTACGATCT TGGCTCAGTG CAACCTCGC CTCTGGGTT CAAGCGATT C

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3401 TGCTGCCTCA GCCTCGGAG TAGCTGGGAT TGCAGGCACC GCGCACCAG

3451 CCTGGCTAAT TTTTGATTTT TTAGTAGAGA CGGGGTTTCA CCATGTTGGT

3501 CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT GGCCTCGAA

3551 AGTGCTGGGa tatacaaggc GTGAGCCACC AGCCAGGCCA AGATATTNTT

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3601 NTAAAGNNAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA
 3651 GTAACATNG GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG
 3701 ACAGCCATAG TATAGTGTGT CACTOGTGGT TGGTGTCTT TCATGGTCT
 3751 GCCCTGTCAA AGGTCCCTAT TTGAAATGTG TTATAATACA AACAAAGGAAG
 3801 CACATTGTGT ACAAATACT TATGTATTTA TGAATCCATG ACCAAATTAA
 3851 ATATGAAACC TTATATAAAA AAAAAAAAAA A

A nucleotide sequence of a human TR6. (SEQ ID NO: 1).

Table 2^h

1	Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys	16
17	Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro	32
33	Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu	48
49	Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln	64
65	Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu	80
81	Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser	96
97	Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe	112
113	Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro	128
129	Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe	144
145	Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys	160
161	Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile	176
177	Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala	192
193	Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp	208

5	209	Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly	224
10	225	Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp	240
15	241	Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro	256
20	257	Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn	272
25	273	Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala	288
30	289	Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp	304
35	305	Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val	320
40	321	Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp	336
45	337	Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr	352
50	353	Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala	368
55	369	Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu	384
60	385	Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met	400
65	401	Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End	411

An amino acid sequence of a human TR6. (SEQ ID NO: 2).

One polynucleotide of the present invention encoding TR6 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human of human thymus stromal cells, monocytes, peripheral blood lymphocytes, primary dendritic, and bone marrow cells using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) 355:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding TR6 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 94 to 1329 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TR6 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding TR6 variants comprising the amino acid sequence

of TR6 polypeptide of Table 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3^e

1	ATGACCTCCT	TTTCTGCTTG	CGCTGCACCA	GGTGTGATTC	AGGTGAAGTG
51	GAGCTAAGTC	CCTGCACCAC	GACCAGAAAC	ACAGTGTGTC	AGTGCGAAGA
101	AgGCACCTTC	CGGGAAGAAG	ATTCTCCTGA	GATGTGCCGG	AAGTGCCGCA
151	CAGGGTGTCC	CagAGGGATG	GTCAAGGTCG	GTGATTGTAC	ACCCTGGAGT
201	GACATCGAAT	GTGTCCACAA	AGAATCAGGC	ATCATCATAg	GAGTCACAGT

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251 TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTCaAg TCTTTACTGT
301 GGAAGAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG
351 GACCCTGAGC GTGTGGACAG AAGcTCACAA CGACcTGGGG CTGAGGACAA
401 TGTCTCAAT GAGATCGTGA GTATCTTGCA GCCCACCCAG GTCCCTGAGC
451 AGGAAATGGA AGTCCAGGAG CCAGCAGAGC CAACAGGTGT CAACATGTTG
501 TCCCCCGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG CTGAAAGGTC
551 TCAGAGGAGG AGGCTGCTGG TTCCAGCAA TGAAGGTGAT CCCACTGAGA
601 CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC
651 TGGGAgCCgC TCATGAGGAA GTTGGGCCTC ATGGACAATg AGATaaGGT
701 GGCTAAAGCT GAGGCAGCGG GCCACAGGGA CACCTTGTA ACGATGCTGA
751 TAAAGTGGGT CAACAAAACC GGGCGAGATG CCTCTGTCCA CACCCTGCTG
801 GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA AGATTGAGGA
851 CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT
901 CTGCCATGTC CTAAGTGTA TTCTCTCAG GAAGTCAGAC CTCCCTGGT
951 TTACCTTTT TCTGGAAAAA GCCCACTGG ACTCCAGTCA GTAGGAAAGT
1001 GCCACAATTG TCACATGACC GGTACTGGAA GAACTCTCC CATCCAACAT
1051 CACCCAGTGG AT

c A partial nucleotide sequence of a human TR6. (SEQ ID NO: 3).

Table 4^d

1 DLLFCLRCTR CDSGEVELSP CTTTRNTVCQ CEEGTFREED SPEMCRKCRT

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51  GCPRGMVKVG DCTPWSDIEC VHKEGIIIG VTVAAVVLIV AVFVCKSLIW
101 KKVLPLYLGI CSGGGGDPER VDRSSQRPGA EDNVLNEIVS ILQPTQVPEQ
151 EMEVQEPAEP TGVNMLSPGE SEHLLPEAEA ERSQRRRLV PANEGDPTET
201 LRQCFFDFAD LVPFDSWEPL MRKLGLMDNE IKVAKAEAAG HRDTLYTMLI
251 KWNKTGRDA SVHTLLDALE TLGERLAKQK IEDHLLSSGK FMYLEGNADS
301 AMS*

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A partial amino acid sequence of a human TR6. (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, including that of SEQ ID NO:3, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TR6 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the TR6 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding TR6 polypeptide comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID

NO: 3, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, TR6 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID NO:3. Also included with TR6 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran

mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, CQS, HeLa, C127, 3T3, BHK, HEX 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TR6 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TR6 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TR6 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TR6 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TR6 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TR6. Individuals carrying mutations in the TR6 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TR6 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TR6 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, through detection of mutation in the TR6 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease

syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of TR6 polypeptide or TR6 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TR6, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The 3' untranslated region of TR6 matches the 295 bp nucleotide sequence of a mapped EST (Genbank ID: D20151). This EST has been mapped by the Whitehead Institute to chromosome 8, 97.68 cR from the top of the Chromosome 8 linkage group

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TR6 polypeptides. The term "immunospecific" means that the antibodies have substantial greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TR6 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TR6 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TR6 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TR6 polypeptide via a vector directing expression of TR6 polynucleotide *in vivo* in order to induce such an immunological response to pro-

duce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TR6 polypeptide wherein the composition comprises a TR6 polypeptide or TR6 gene. The vaccine formulation may further comprise a suitable carrier. Since TR6 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

We have now discovered that TL2 of SEQ ID NO: 5 (otherwise known as TRAIL, Immunity (6):673-682 (1995)) is a ligand of TR6. Thus, the TR6 polypeptide of the present invention, and one of its ligands, TL2 may be employed in a screening process for compounds which bind the receptor, or its ligand, and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention, or its ligand TL2. Thus, polypeptides of the invention may be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TR6 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate TR6 on the one hand and which can inhibit the function of TR6 or remove TR6 expressing cells on the other hand. Antagonists, or agents which remove TR6 expressing cells, may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease. Agonists can be employed for therapeutic and prophylactic purposes for such conditions responsive to activation of T cells and other components of the immune system, such as for treatment of cancer and AIDS. However, agonists can also be employed for inappropriate stimulation of T cells and other components of the immune system which leads to down modulation of immune activity with therapeutic or prophylactic application for conditions such as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, Bone diseases, atherosclerosis, and Alzheimers disease.

Candidate compounds may be identified using assays to detect compounds which inhibit binding of TL2 to TR6 in either cell-free or cell based assays. Suitable cell-free assays may be readily determined by one of skill in the art. For example, an ELISA format may be used in which purified TR6, or a purified derivative of TR6, containing the extracellular domain of TR6, is immobilized on a suitable surface, either directly or indirectly (e.g., via an antibody to TR6) and candidate compounds are identified by their ability to block binding of purified TL2 to TR6. The binding of TL2 to TR6 could be detected by using a label directly or indirectly associated with TL2. Suitable detection systems include the streptavidin horseradish peroxidase conjugate, or direct conjugation by a tag, e.g., fluorescein. Conversely, purified TL2 may be immobilized on a suitable surface, and candidate compounds identified by their ability to block binding of purified TR6 to TL2. The binding of TR6 to TL2 could be detected by using a label directly or indirectly associated with TR6. Many other assay formats are possible that use the TR6 protein and its ligands.

Suitable cell based assays may be readily determined by one of skill in the art. In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a known ligand, such as TL2, or test compound to observe binding, or stimulation or inhibition of a functional response. The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor, such as the ligand TL2. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor or its ligand (e.g. TL2) using detection systems appropriate to the cells bearing the receptor or its ligand.

and fusion proteins thereof at their surfaces. Typical fusion partners include fusing the extracellular domain of the receptor or ligand with the intracellular tyrosine kinase domain of a second receptor. Inhibitors of activation are generally assayed in the presence of a known agonist, such as the ligand TL2, and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Examples of potential TR6 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the TR6, e.g., a fragment of the ligand TL2, or small molecules which bind to the receptor, or its ligand, but do not elicit a response, so that the activity of the receptor is prevented. Examples of potential TR6 agonists include antibodies that bind to TR6, its ligand, such as TL2, or derivatives thereof, and small molecules that bind to TR6. These agonists will elicit a response mimicking all or part of the response induced by contacting the native ligand.

The nucleotide sequence of TL2 (SEQ ID NO:5) (published by Immunex Research and Development Corporation, Seattle, Washington as TNF-related apoptosis-inducing ligand (TRAIL) TWiley SR, *et al.* Immunity (6):673-682 (1995)) is as follows:

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1 CCTCACTGAC TATAAAAGAA TAGAGAAGGA AGGGCTTCAG TGACCGGCTG
51 CCTGGCTGAC TTACAGCAGT CAGACTCTGA CAGGATCATG GCTATGATGG
101 AGGTCCAGGG GGGACCCAGC CTGGGACAGA CCTGCGTGCT GATCGTGATC
151 TTCACAGTGC TCCTGCAGTC TCTCTGTGTG GCTGTAACCT ACGTGTACTT
201 TACCAACGAG CTGAAGCAGA TGCAGGACAA GTACTCCAAA AGTGGCATTG
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251 CTTGTTTCTT AAAAGAAGAT GACAGTTATT GGGACCCCAA TGACGAAGAG

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301 AGTATGAACA GCCCCTGCTG GCAAGTCAAG TGGCAACTCC GTCAGCTCGT

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351 TAGAAAGATG ATTTTGAGAA CCTCTGAGGA AACCATTCTT ACAGTTCAAG

20

401 AAAAGCAACA AAATATTTCT CCCCTAGTGA GAGAAAGAGG TCCTCAGAGA

25

451 GTAGCAGCTC ACATAACTGG GACCAGAGGA AGAAGCAACA CATTGTCTTC

30

501 TCCAAACTCC AAGAATGAAA AGGCTCTGGG CCGCAAATA AACTCCTGGG

35

551 AATCATCAAG GAGTGGGCAT TCATTCTGA GCAACTTGCA CTTGAGGAAT

40

601 GGTGAAGTGG TCATCCATGA AAAAGGTTT TACTACATCT ATTCCCAAAC

45

651 ATACTTTTGA TTTCAGGAGG AAATAAAGA AAACACAAAG AACGACAAAC

50

701 AAATGGTCCA ATATATTTAC AAATACACAA GTTATCCTGA CCCTATATTG

751 TTGATGAAAA GTGCTAGAAA TAGTTGTTGG TCTAAAGATG CAGAATATGG

801 ACTCTATTCC ATCTATCAAG GGGGAATATT TGAGCTTAAG GAAAATGACA

851 GAATTTTGT TTCTGTAACA AATGAGCACT TGATAGACAT GGACCATGAA

901 GCCAGTTTTT TCGGGGCCTT TTTAGTTGGC TAACTGACCT GGAAAGAAAA

951 AGCAATAACC TCAAAGTGAC TATTCAGTTT TCAGGATGAT ACACTATGAA

1001 GATGTTTCAA AAAATCTGAC CAAAACAAAC AAACAGAAAA CAGAAAACAA

1051 AAAAACCTCT ATGCAATCTG AGTAGAGCAG CCACAACCAA AAAATTCTAC

1101 AACACACACT GTTCTGAAAG TGACTCACTT ATCCCAAGAA AATGAAATTG

1151 CTGAAAGATC TTTCAGGACT CTACCTCATA TCAGTTTGCT AGCAGAAATC

1201 TAGAAGACTG TCAGCTTCCA AACATTAATG CAATGGTTAA CATCTTCTGT

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1251 CTTTATAATC TACTCCTTGT AAAGACTGTA GAAGAAAGCG CAACAATCCA
 1301 TCTCTCAAGT AGTGTATCAC AGTAGTAGCC TCCAGGTTTC CTTAAGGGAC
 1351 AACATCCTTA AGTCAAAAGA GAGAAGAGGC ACCACTAAAA GATCGCAGTT
 1401 TGCCTGGTGC AGTGGCTCAC ACCTGTAATC CCAACATTTT GCGAACCCTAA
 1451 GGTGGGTAGA TCACGAGATC AAGAGATCAA GACCATAGTG ACCAACATAG
 1501 TGAAACCCCA TCTCTACTGA AAGTGCAAAA ATTAGCTGGG TGTGTTGGCA
 1551 CATGCCTGTA GTCCAGCTA CTGAGAGGC TGAGGCAGGA GAATCGTTTG
 1601 AACCCGGGAG GCAGAGGTTG CAGTGTGGTG AGATCATGCC ACTACACTCC
 1651 AGCCTGGCGA CAGAGCGAGA CTGTTTCA AAAAAAAAAA AAAAAAAAAA
 1701 CTTAGTAAG TACGTGTTAT TTTTTCAT AAAATTCTAT TACAGTATGT
 1751 CAAAAAAAAA AAAAAAAAAA

The amino acid sequence of TL2 (SEQ ID NO:6) (published by Immunex Research and Development Corporation, Seattle, Washington as TNF-related apoptosis-inducing ligand (TRAIL) TWiley SR, *et al.* *Immunity* (6):673-682 (1995)) is as follows:

1 Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys 16
 17 Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala 32
 33 Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys 48
 49 Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr 64
 65 Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val 80
 81 Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser 96

	97	Glu	Glu	Thr	Ile	Ser	Thr	Val	Gln	Glu	Lys	Gln	Gln	Asn	Ile	Ser	Pro	112
5	113	Leu	Val	Arg	Glu	Arg	Gly	Pro	Gln	Arg	Val	Ala	Ala	His	Ile	Thr	Gly	128
	129	Thr	Arg	Gly	Arg	Ser	Asn	Thr	Leu	Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	144
10	145	Lys	Ala	Leu	Gly	Arg	Lys	Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	160
	161	His	Ser	Phe	Leu	Ser	Asn	Leu	His	Leu	Arg	Asn	Gly	Glu	Leu	Val	Ile	176
15	177	His	Glu	Lys	Gly	Phe	Tyr	Tyr	Ile	Tyr	Ser	Gln	Thr	Tyr	Phe	Arg	Phe	192
	193	Gln	Glu	Glu	Ile	Lys	Glu	Asn	Thr	Lys	Asn	Asp	Lys	Gln	Met	Val	Gln	208
20	209	Tyr	Ile	Tyr	Lys	Tyr	Thr	Ser	Tyr	Pro	Asp	Pro	Ile	Leu	Leu	Met	Lys	224
	225	Ser	Ala	Arg	Asn	Ser	Cys	Trp	Ser	Lys	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	240
25	241	Ser	Ile	Tyr	Gln	Gly	Gly	Ile	Phe	Glu	Leu	Lys	Glu	Asn	Asp	Arg	Ile	256
	257	Phe	Val	Ser	Val	Thr	Asn	Glu	His	Leu	Ile	Asp	Met	Asp	His	Glu	Ala	272
30	273	Ser	Phe	Phe	Gly	Ala	Phe	Leu	Val	Gly	End							281

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, related to both an excess of and insufficient amounts of TR6 activity.

If the activity of TR6 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the TR6, or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of TR6 polypeptides still capable of binding the ligand in competition with endogenous TR6 may be administered. Typical embodiments of such competitors comprise fragments of the TR6 polypeptide.

In still another approach, expression of the gene encoding endogenous TR6 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TR6 and its activity, several approaches are also

available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TR6, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TR6 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of TR6 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of TR6 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 $\mu\text{g/kg}$ of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

Two ESTs (EST#1760054 and EST#1635744) with sequence similarity to the human TNF receptor were discovered in a commercial EST database. Analysis of the two nucleotide sequences (3,466 bp and 2,641 bp respectively), revealed each was a partial sequence of the complete cDNA sequence, overlapping, with 100% identity, 2,226 bp at the nucleotide level. Together, the two sequences encompassed the complete predicted cDNA sequence of 3,881 bp, and encoded an open reading frame for a novel member of the TNF receptor superfamily and named TR6. The predicted protein is 411 amino acids long with a hydrophobic membrane spanning region indicating that at least one form of TR6 is expressed as a membrane bound protein. Comparison of TR6 protein sequence, with other TNF receptor family proteins indicates that it has two of the cysteine-rich repeats characteristic of the extracellular domains of this family, and an intracellular death domain,

Northern blot of TR6.

Various tissues and cell lines were screened for mRNA expression by Northern blot. RNA was prepared from cells and cell lines using Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH), run in denaturing agarose gels (Sambrook et al., Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Lab Press, NY (1989)) and transferred to Zeta-probe nylon membrane (Biorad, Hercules, CA.) via vacuum blotting in 25mM NaOH for 90 min. After neutralization for 5-10 minutes with 1M tris-HCl, pH 7.5 containing 3M NaCl, the blots were prehybridized with 50% formamide, 8% dextran sulfate, 6XSSPE, 0.1%SDS and 100mg/ml of sheared and denatured salmon sperm DNA for at least 30 min. At 42°C. cDNA probes were labeled with 32P-CTP by random priming (Statagene, La Jolla, CA), briefly denatured with 0.25M NaOH and added to the prehybridization solution. After a further incubation for at least 24h at 42°C, the blots were washed in high stringency conditions and exposed to X-ray film.

Very high expression of TR6 RNA was detected in aortic endothelial cells. High expression was also detected in monocytes. Low expression was detected in bone marrow and CD4+ activated PBLs. Very low, but detectable levels of TR6 RNA was expressed in CD19+ PBLs, CD8+ PBLs (both activated and unstimulated), and unstimulated CD4+ PBLs.

In hematopoietic cell lines, low levels of TR6 RNA was expressed in HL60 (promyelocyte), KG1a (promyeloblast) and KG1 (myeloblast) cell lines. Very low but detectable levels of TR6 RNA was expressed in U937 (monoblast) and THP-1 (monocyte) cell lines.

The major RNA form is 3.8 kb in size.

SEQUENCE LISTING

5

(1) GENERAL INFORMATION

10

(i) APPLICANT: SmithKline Beecham Corporation

15

(ii) TITLE OF THE INVENTION: TUMOR NECROSIS FACTOR RELATED
RECEPTOR, TR6

20

(iv) CORRESPONDENCE ADDRESS

(A) ADDRESSEE: SmithKline Beecham,
Corporate Intellectual Property

25

(B) STREET: Two New Horizons Court

(C) CITY: Brentford

(D) COUNTY: Middlesex

(E) COUNTRY: United Kingdom

30

(F) POST CODE: TW8 9EP

35

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER IBM Compatible

(C) OPERATING SYSTEM: DOS

40

(D) SOFTWARE: FastSEQ for Windows Version 2.0

45

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER TO BE ASSIGNED

(B) FILING DATE: 22-AUGUST-1997

(C) CLASSIFICATION: Unknown

50

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER 08/853,684

(B) FILING DATE: 09-MAY-1997

55

(viii) ATTORNEY/AGENT INFORMATION

- (A) NAME: THOMPSON, Clive Beresford
 (B) GENERAL AUTHORISATION NUMBER 5630
 (C) REFERENCE/DOCKET NUMBER GH-50008-1

(ix) TELECOMMUNICATION INFORMATION

- (A) TELEPHONE: +44 181 975 6347
 (B) TELEFAX: +44 181 975 6294
 (C) TELEX:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 3,881 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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    GCOGCTTOGG GGGCCCGGAA AAGGCAAGGC CCAGGACCCA GGGAGGGGOG GGGAGCCAGG      180
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    TCAGCTGAGT CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA      300
    CAACAAAAGA GGTCCAGCCC CTGAGAGGGA TTGTGTCCAC CTGGACACCA TATCTCAGAA      360
    GAOGGTAGAG ATTGCATCTC CTGCAAATAT GGACAGGACT ATAGCACTCA ATGGAATGAC      420
45  CTCCTTTTCT GCTTGGCTG CACCAGGTGT GATTGAGTG AAGTGGAGCT AAGTCCCTGC      480
    ACCACGACCA GAAACACAGT GTGTGAGTGC GAAGAAGGCA CCTTCGGGA AGAAGATTCT      540
    CCTGAGATGT GCGGAAGTG CGCACAGGG TGTCCCAGAG GGATGGTCAA GGTGGGTGAT      600
50  TGTACACCCT GGAGTGACAT OGAATGTGTC CACAAAGAAT CAGGCATCAT CATAGGAGTC      660
    ACAGTTGCAG CGTAGTCTT GATTGTGGCT GTGTTTGTTC GCAAGTCTT ACTGTGGAAG      720
    AAAGTCCTTC CTTACCTGAA AGGCATCTGC TCAGGTGGTG GTGGGGACCC TGAGGTGTG      780

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 10 AGGAAGTTGG GCCTCATGGA CAATGAGATA AAGGTGGCTA AAGCTGAGGC AGCGGGCCAC 1140
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5 GTTTTGTTCC GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATCACACATT 3000
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 15 OGGGGTTTCA CCATGTTGGT CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT 3540
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 20 CACTCGTGGT TGGTGTCTT TCATGGTCT GCGCTGTCAA AGGTCCCTAT TTGAAATGTG 3780
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(2) INFORMATION FOR SEQ ID NO: 2:

30 (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 411 amino acids
 (B) TYPE: amino acid
 35 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

45 Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
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 50 Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
 35 40 45
 Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln

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	50		55		60
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	Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser				
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	Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe				
10		100		105	110
	Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro				
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	Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe				
15		130		135	140
	Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys				
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		165		170	175
20					
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	Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp				
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25					
	Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly				
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	Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp				
	225		230		235 240
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	Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn				
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	Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp				
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	Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val				
	305		310		315 320
	Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp				
		325		330	335
45					
	Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr				
		340		345	350
	Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala				
		355		360	365
50					
	Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu				
		370		375	380
	Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met				

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	CTCTGAGACA	GTGCTT OGAT	GACTTTGCAG	ACTTGGTGCC	CTTTGACTCC	TGGGAGCOGC	660
	TCATGAGGAA	GTGGGGCCTC	ATGGACAATG	AGATAAAGGT	GGCTAAAGCT	GAGGCAGCGG	720
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40	CCTCTGTCCA	CACCCTGCTG	GATGCCTTGG	AGACGCTGGG	AGAGAGACTT	GCCAAGCAGA	840
	AGATTGAGGA	CCACTTGTTG	AGCTCTGGAA	AGTTCATGTA	TCTAGAAGGT	AATGCAGACT	900
	CTGCCATGTC	CTAAGTGTGA	TTCTCTTCAG	GAAGTCAGAC	CTTCCCTGGT	TTACCTTTTT	960
	TCTGGAAAAA	GCCCAACTGG	ACTCCAGTCA	GTAGGAAAGT	GCCACAATTG	TACATGACC	1020
45	GGTACTGGAA	GAAACTCTCC	CATCCAACAT	CACCCAGTGG	AT		1062

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

10

Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
 1 5 10 15

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15

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Arg Thr Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro
 50 55 60

20

Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly
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Val Thr Val Ala Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys
 85 90 95

25

Ser Leu Leu Trp Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser
 100 105 110

Gly Gly Gly Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro
 115 120 125

30

Gly Ala Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro
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Thr Gln Val Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro
 145 150 155 160

35

Thr Gly Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu
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Pro Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
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40

Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
 195 200 205

Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu
 210 215 220

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Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly
 225 230 235 240

His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr
 245 250 255

50

Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu
 260 265 270

Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser

55

275 280 285
 Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1769 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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 TTACAGCAGT CAGACTCTGA CAGGATCATG GCTATGATGG AGGTCCAGGG GGGACCCAGC 120
 CTGGGACAGA CTGCGTGCT GATCGTGATC TTCACAGTGC TCCTGCAGTC TCTCTGTGTG 180
 GCTGTAACCTT ACGTGTACTT TACCAACGAG CTGAAGCAGA TGCAGGACAA GTACTCCAAA 240
 25 AGTGGCATTG CTTGTTTCTT AAAAGAAGAT GACAGTTATT GGGACCCCAA TGACGAAGAG 300
 AGTATGAACA GCCCCTGCTG GCAAGTCAAG TGGCAACTCC GTCAGCTCGT TAGAAAGATG 360
 ATTTTGAGAA CCTCTGAGGA AACCATTCTT ACAGTTCAAG AAAAGCAACA AAATATTTCT 420
 CCCCTAGTGA GAGAAAGAGG TCCTCAGAGA GTAGCAGCTC ACATAACTGG GACCAGAGGA 480
 30 AGAAGCAACA CATTGTCTTC TCCAACTCC AAGAATGAAA AGGCTCTGGG CCGCAAAATA 540
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 35 AAATACACAA GTTATCCTGA CCCTATATTG TTGATGAAAA GTGCTAGAAA TAGTTGTTGG 780
 TCTAAAGATG CAGAATATGG ACTCTATTCC ATCTATCAAG GGGGAATATT TGAGCTTAAG 840
 GAAAATGACA GAATTTTGTG TTCTGTAACA AATGAGCACT TGATAGACAT GGACCATGAA 900
 GCCAGTTTTT TCGGGGCCTT TTTAGTTGGC TAACTGACCT GGAAAGAAAA AGCAATAACC 960
 40 TCAAAGTGAC TATTCAGTTT TCAGGATGAT ACACTATGAA GATGTTTCAA AAAATCTGAC 1020
 CAAAACAAAC AAACAGAAAA CAGAAAACAA AAAAACCTCT ATGCAATCTG AGTAGAGCAG 1080
 CCACAACCAA AAAATTCTAC AACACACACT GTTCTGAAAG TGACTCACTT ATCCCAAGAA 1140
 AATGAAATTG CTGAAAGATC TTTCAGGACT CTACCTCATA TCAGTTTGCT AGCAGAAATC 1200
 45 TAGAAGACTG TCAGCTTCCA AACATTAATG CAATGGTTAA CATCTTCTGT CTTTATAATC 1260
 TACTCCTTGT AAAGACTGTA GAAGAAAGCG CAACAATCCA TCTCTCAAGT AGTGTATCAC 1320
 AGTAGTAGCC TCCAGGTTTC CTTAAGGGAC AACATCCTTA AGTCAAAAGA GAGAAGAGGC 1380
 ACCACTAAAA GATCGCAGTT TGCTGGTGC AGTGGCTCAC ACCTGTAATC CCAACATTTT 1440
 50 GGGAAACCCAA GGTGGGTAGA TCACGAGATC AAGAGATCAA GACCATAGTG ACCAACATAG 1500
 TGAAACCCCA TCTCTACTGA AAGTGCAAAA ATTAGCTGGG TGTGTTGGCA CATGCCTGTA 1560
 GTCCCAGCTA CTTGAGAGGC TGAGGCAGGA GAATCGTTTG AACCCGGGAG GCAGAGGTTG 1620

CAGTGTGGTG AGATCATGCC ACTACACTCC AGCCTGGCGA CAGAGCGAGA CTTGGTTTCA 1680
 AAAAAAAAAA AAAAAAAAAA CTTCAAGTAAG TACGTGTTAT TTTTTCAT AAAATTCTAT 1740
 TACAGTATGT CAAAAAAAAA AAAAAAAAAA 1769

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 281 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys
 1 5 10 15
 Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala
 20 25 30
 Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys
 35 40 45
 Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr
 50 55 60
 Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val
 65 70 75 80
 Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser
 85 90 95
 Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro
 100 105 110
 Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly
 115 120 125
 Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu
 130 135 140
 Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly
 145 150 155 160
 His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile
 165 170 175
 His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe
 180 185 190
 Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln
 195 200 205
 Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys

210 215 220
 Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
 5 225 230 235 240
 Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile
 245 250 255
 Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala
 10 260 265 270
 Ser Phe Phe Gly Ala Phe Leu Val Gly
 275 280

Claims

- 20 1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence.
- 25 2. The polynucleotide of claim 1 which is DNA or RNA.
3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.
- 30 4. The polynucleotide of claim 3 wherein said nucleotide sequence comprises the TR6 polypeptide encoding sequence contained in SEQ ID NO:1.
5. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.
- 35 6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a TR6 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.
7. A host cell comprising the expression system of claim 6.
- 40 8. A process for producing a TR6 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.
9. A process for producing a cell which produces a TR6 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a TR6 polypeptide.
- 45 10. A TR6 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.
- 50 11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.
12. An antibody immunospecific for the TR6 polypeptide of claim 10.
- 55 13. A method for the treatment of a subject in need of enhanced activity or expression of TR6 polypeptide of claim 10 comprising:
 - (a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or
 - (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80%

identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

5 14. A method for the treatment of a subject having need to inhibit activity or expression of TR6 polypeptide of claim 10 comprising:

- 10 (a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or
 (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor; and/or
 (c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.

15 15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TR6 polypeptide of claim 10 in a subject comprising:

- 20 (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TR6 polypeptide in the genome of said subject; and/or
 (b) analyzing for the presence or amount of the TR6 polypeptide expression in a sample derived from said subject.

16. A method for identifying agonists to TR6 polypeptide of claim 10 comprising:

- 25 (a) contacting a cell which produces a TR6 polypeptide with a candidate compound; and
 (b) determining whether the candidate compound effects a signal generated by activation of the TR6 polypeptide.

17. An agonist identified by the method of claim 16.

30 18. The method for identifying antagonists to TR6 polypeptide of claim 10 comprising:

- 35 (a) contacting said a cell which produces a TR6 polypeptide with an agonist; and
 (b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

19. An antagonist identified by the method of claim 18.

20. A recombinant host cell produced by the process of claim 9 or a membrane thereof expressing a TR6 polypeptide.

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(19)



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(11)

EP 0 870 827 A3

(12)

EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
28.10.1998 Bulletin 1998/44

(51) Int. Cl.⁶: **C12N 15/12**, C07K 14/715,
C07K 16/28, A61K 38/17,
C12Q 1/68, G01N 33/68

(43) Date of publication A2:
14.10.1998 Bulletin 1998/42

(21) Application number: 97310562.0

(22) Date of filing: 23.12.1997

(84) Designated Contracting States:
**AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE**

(30) Priority: 14.03.1997 US 41230 P
09.05.1997 US 853684
22.08.1997 US 916625

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(54) **Tumor necrosis factor related receptor, TR6**

(57) TR6 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR6 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease., among others and diagnostic assays for such conditions.

EP 0 870 827 A3



European Patent
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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P, X	SHERIDAN J P ET AL: "Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors" SCIENCE, vol. 277, 8 August 1997, pages 818-821, XP002075799 * the whole document *	1-12, 15-20	C12N15/12 C07K14/715 C07K16/28 A61K38/17 C12Q1/68 G01N33/68
P, X	PAN G ET AL: "AN ANTAGONIST DECOY RECEPTOR AND A DEATH DOMAIN-CONTAINING RECEPTOR FOR TRAIL" SCIENCE, vol. 277, 8 August 1997, pages 815-818, XP002065147 * the whole document *	1-12, 15, 16, 20	
A	WO 97 01633 A (IMMUNEX CORP) 16 January 1997 * figures 1,2; example 13 *		
A	EP 0 510 691 A (OSAKA BIOSCIENCE INST) 28 October 1992 see the claims * abstract; figures 1,2,7; examples 1,2 *		
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C07K A61K C12N C12Q G01N
INCOMPLETE SEARCH <p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely:</p> <p>Claims searched incompletely:</p> <p>Claims not searched:</p> <p>Reason for the limitation of the search:</p> <p>Although claims 13 and 14 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		28 August 1998	Oderwald, H
CATEGORY OF CITED DOCUMENTS <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

EPO FORM 1503 03.82 (P4-C07)



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Application Number
EP 97 31 0562

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	<p>CHINNAIYAN A M ET AL: "SIGNAL TRANSDUCTION BY DR3, A DEATH DOMAIN-CONTAINING RECEPTOR RELATED TO TNFR-1 AND CD95" SCIENCE, vol. 274, no. 5289, 8 November 1996, pages 990-992, XP000676685</p> <p>* the whole document *</p>		
			<p>TECHNICAL FIELDS SEARCHED (Int.Cl.6)</p>

EPO FORM 1503 03.82 (P04C10)